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C07H 1/00

(52) Domestic classification
C3H B4

(56) Documents cited
GB A 2138821 GB A 2118189

(58) Field of search
C3H

(54) Polynucleotide synthesizing apparatus

(57) A polynucleotide synthesizing apparatus comprises reactor means; reagent and solvent bottle means which is charged with reagents and solvents required for the polynucleotide synthesis reaction; change valve means having at the inlet side thereof a plurality of reagent and solvent ports which are communicated with said reagent and solvent bottles and a common port communicated with said reactor means at the outlet side thereof, said change valve means being adapted to change the passages for communicating each of reagent and solvent bottles means with said reactor means by the rotary operation of a knob; and liquid supply means for supplying reagents and solvents from each of reagent and solvent bottles to said reactor means under pressure of inert gas, said reagent and solvent ports being disposed in order of liquid supply sequence whereby one rotation of said operation knob causes the liquid supply operation in a condensation step.

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The drawing(s) originally filed was (were) informal and the print here reproduced is taken from a later filed formal copy.

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FIG. 1

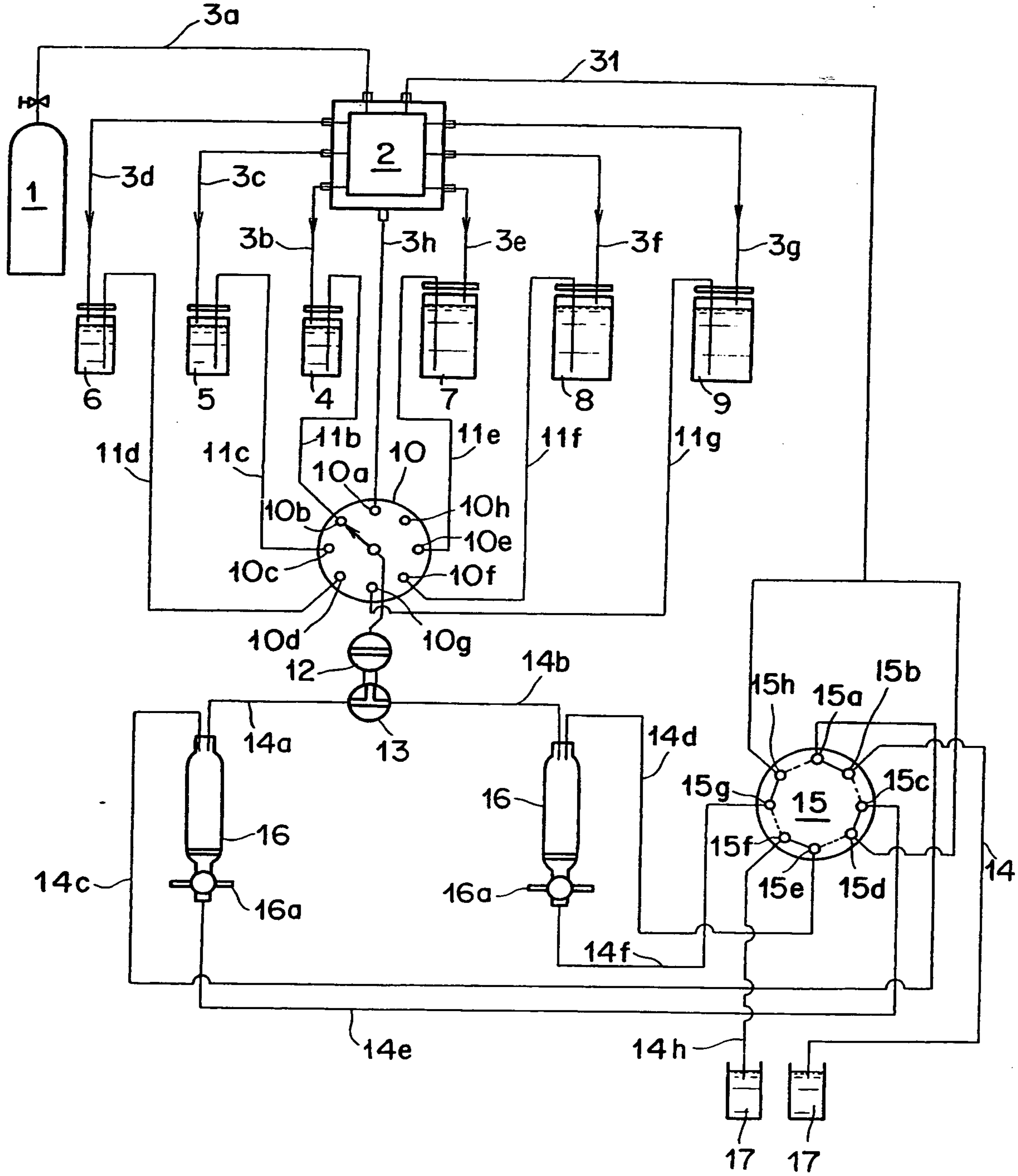
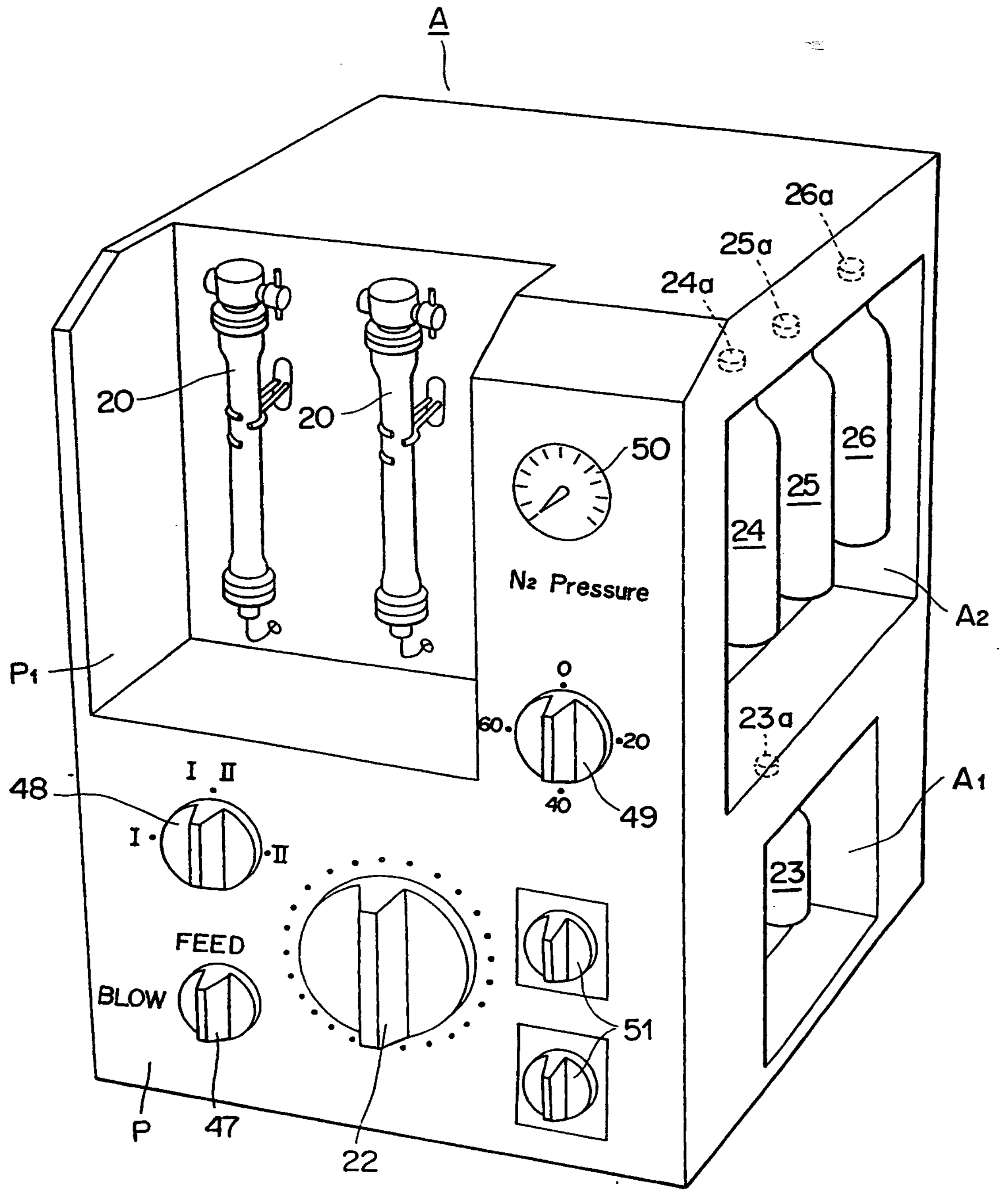


FIG. 2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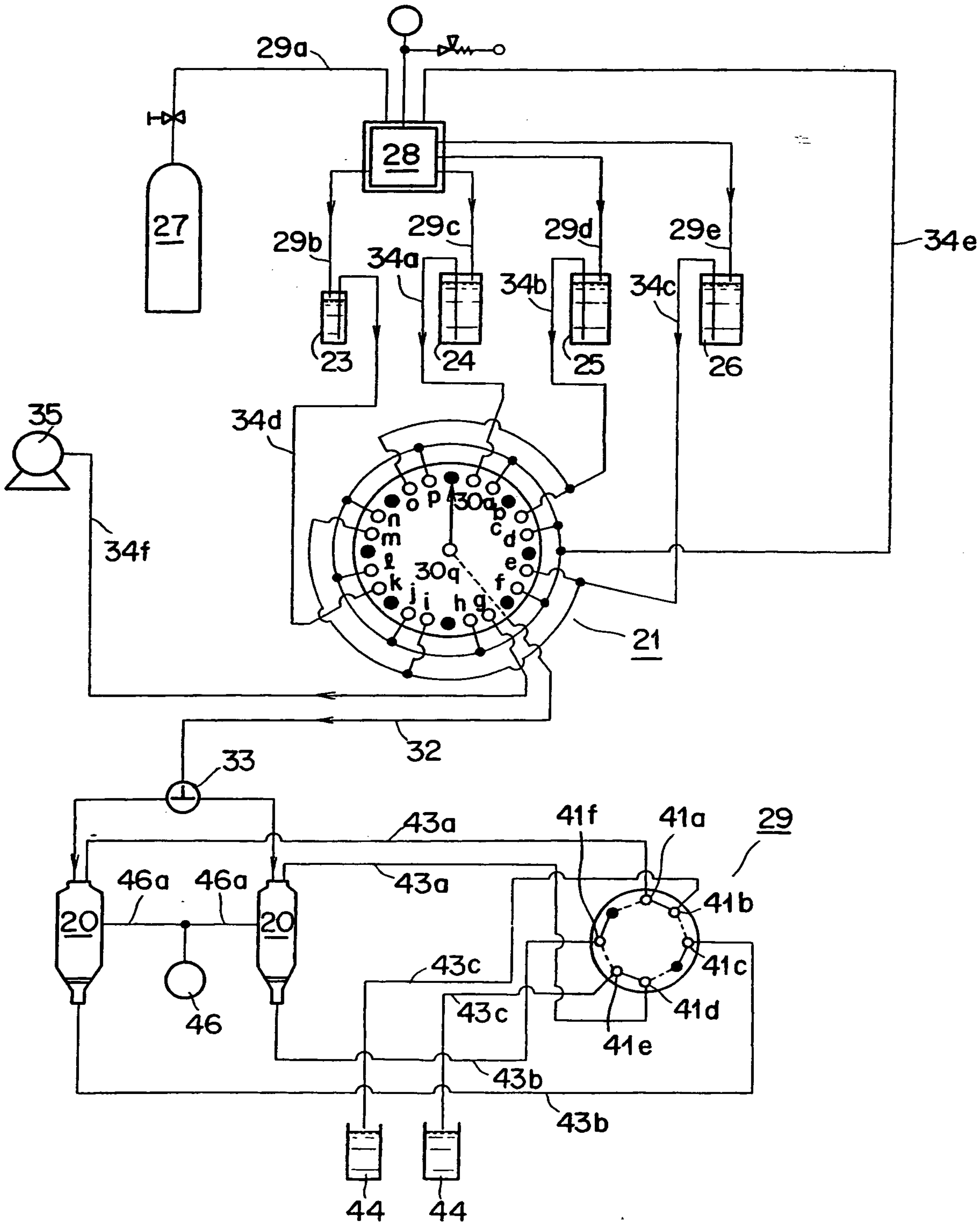
26a
25a
24a
20
20
50
26
25
24
N2 Pressure
A2
P1
0
60
20
40
49
23a
A1
48
I II
I
II
23
FEED
BLOW
51
P
47
22

FIG. 3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o
p
30a
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c
d
n
m
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e
k
30q
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21
32
33
41f
41a
29
43a
46a
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FIG. 4

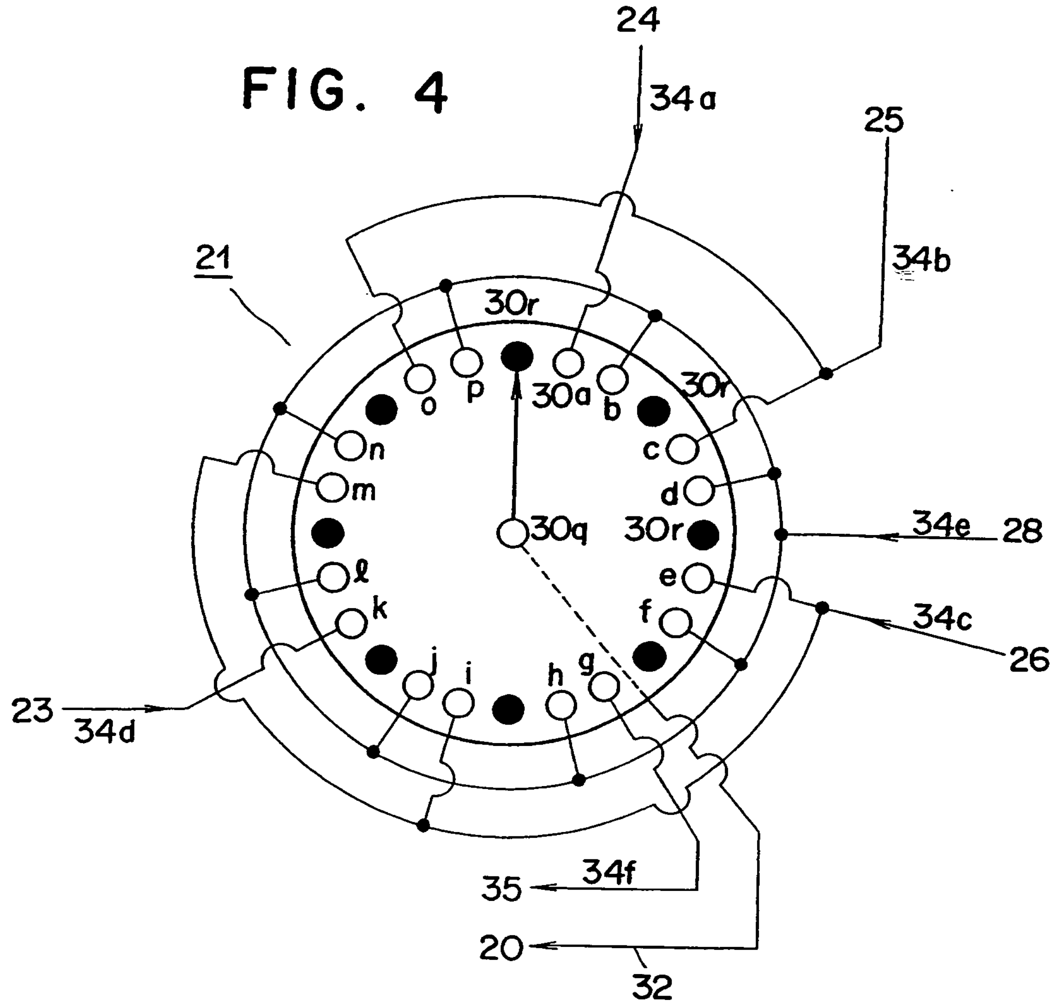


FIG. 5a

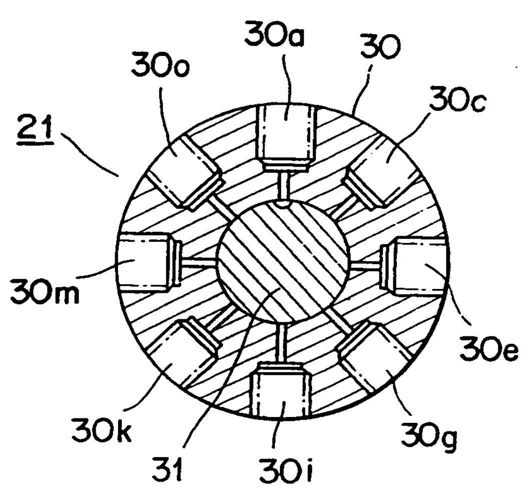


FIG. 6a

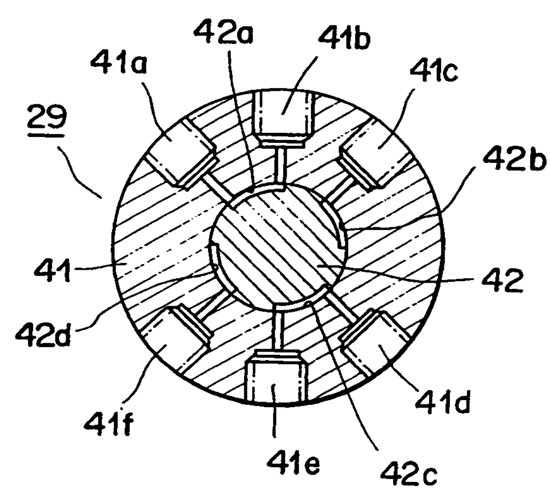


FIG. 5b

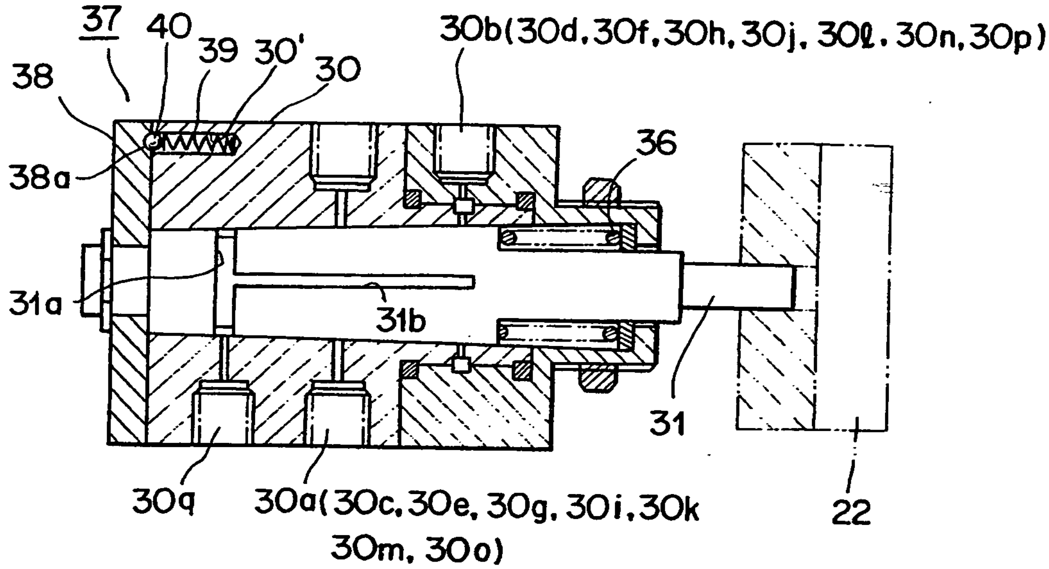


FIG. 6b

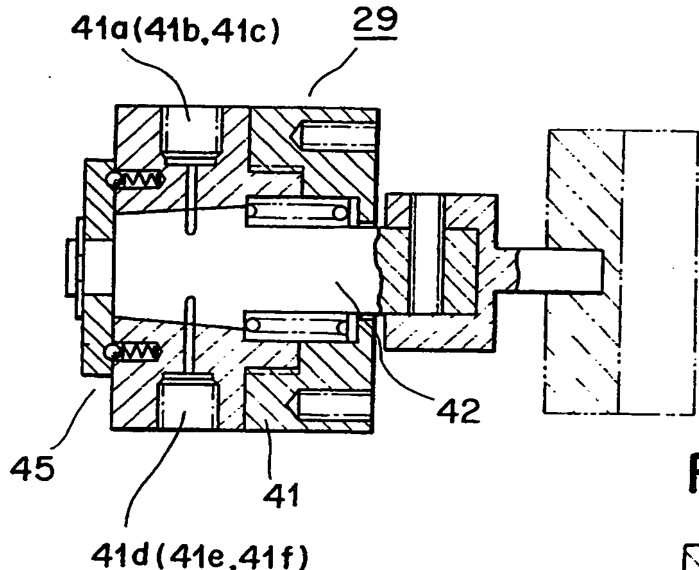


FIG. 8

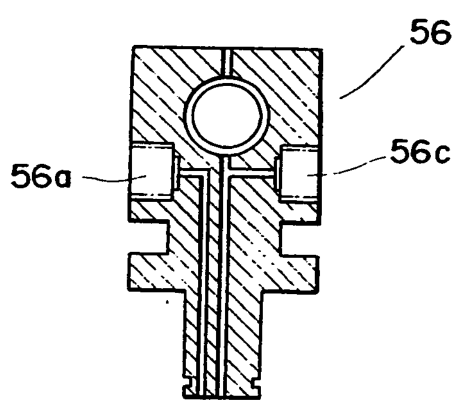
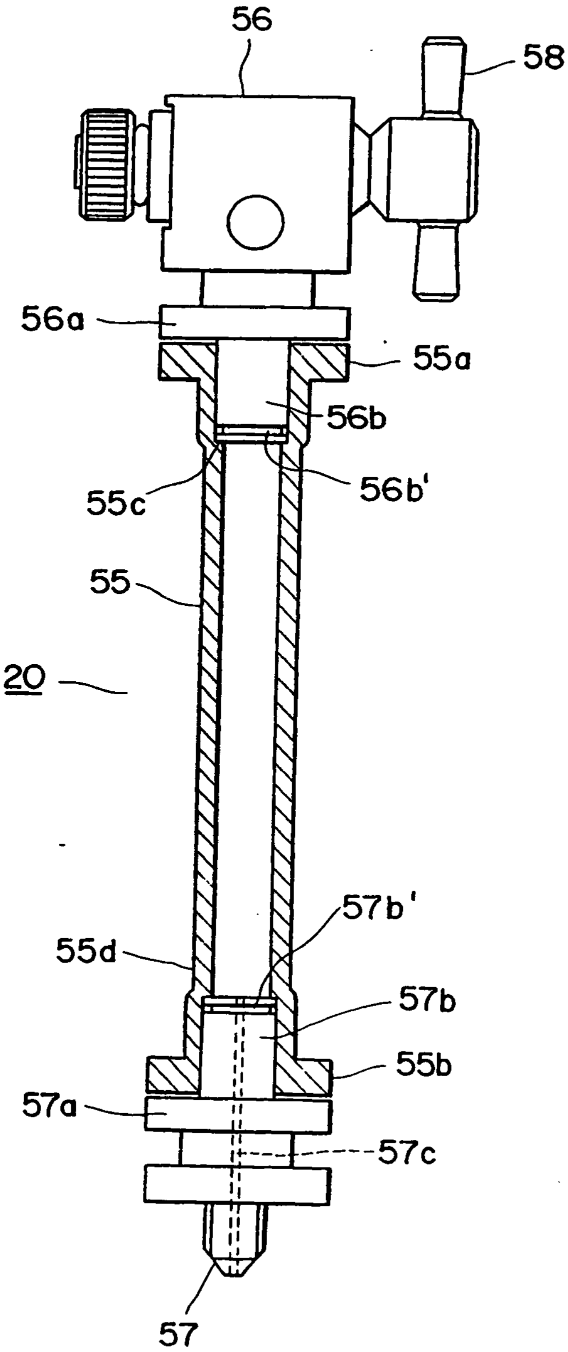


FIG. 7


56
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56b'
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55b
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FIG. 9

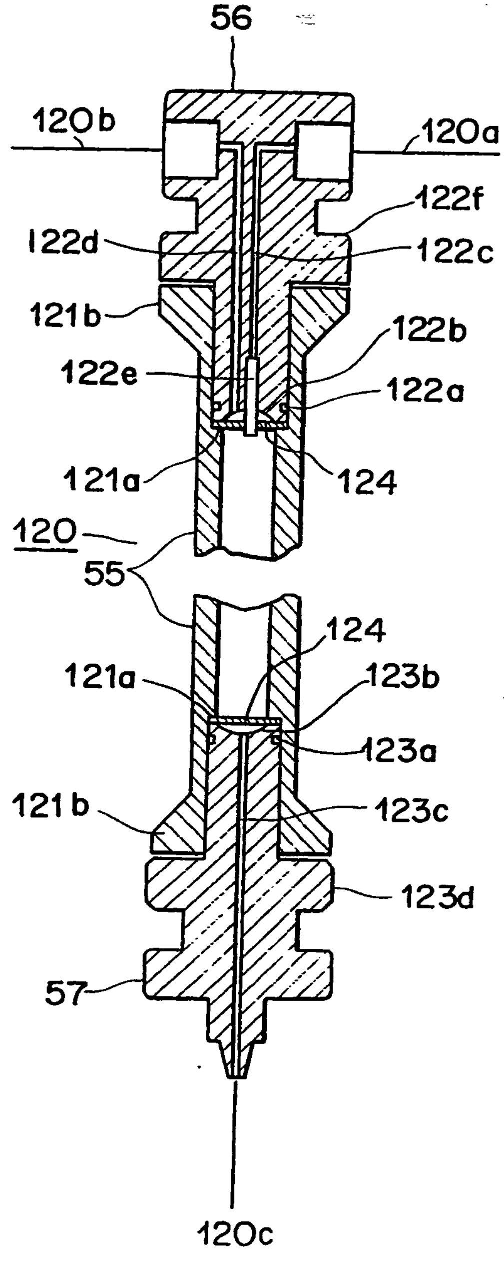
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FIG. 10

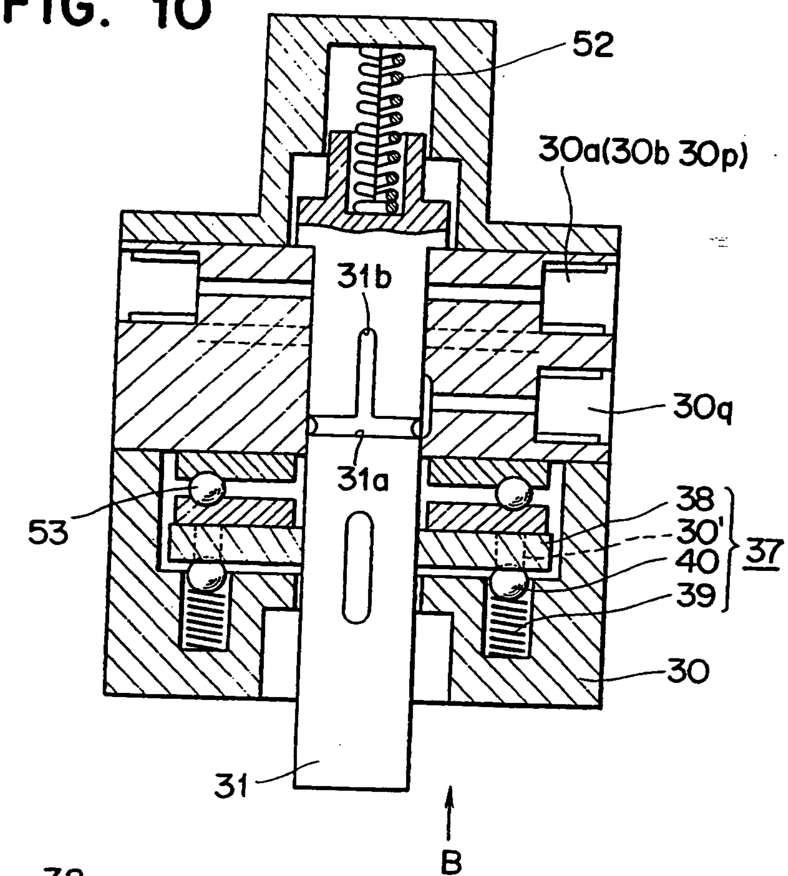
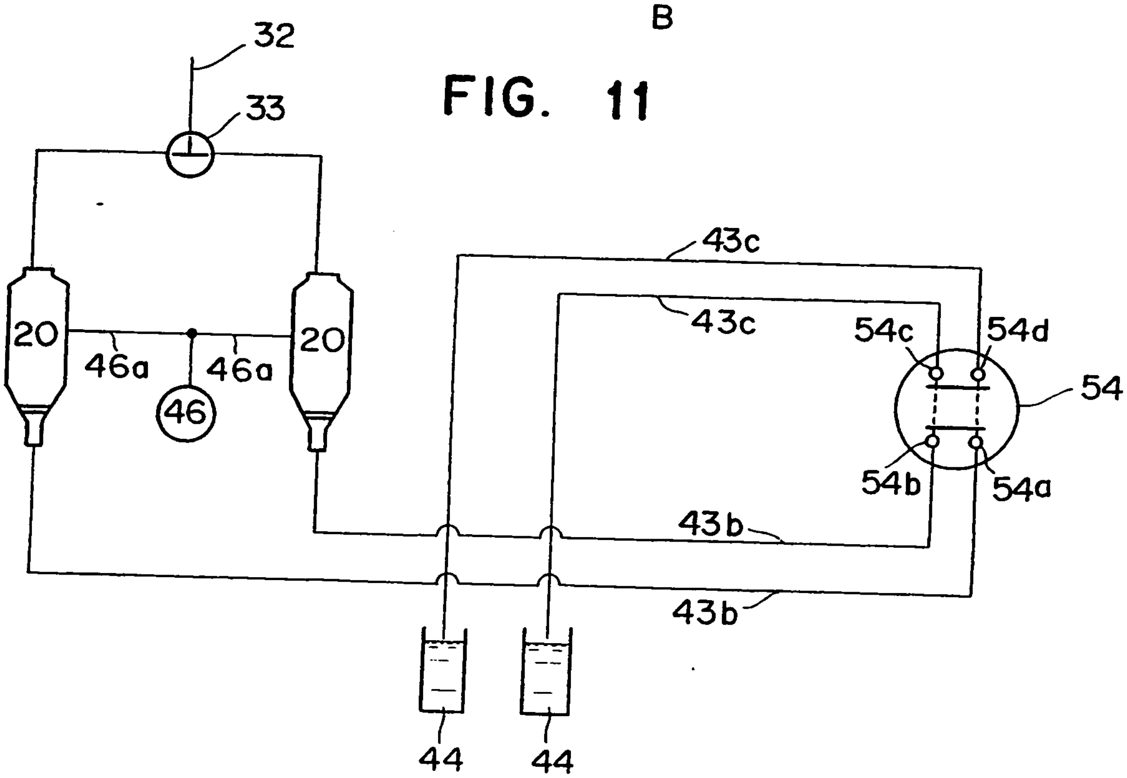


FIG. 11



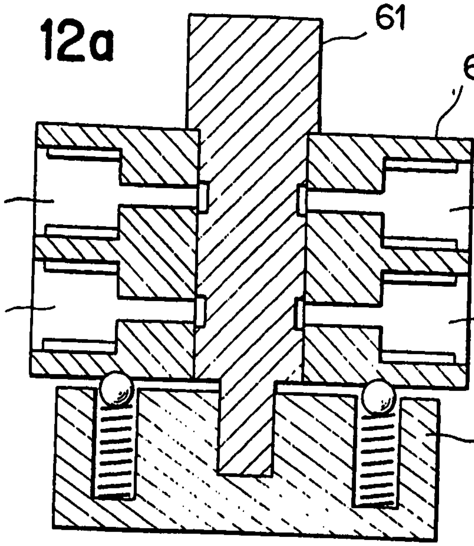


FIG. 12a

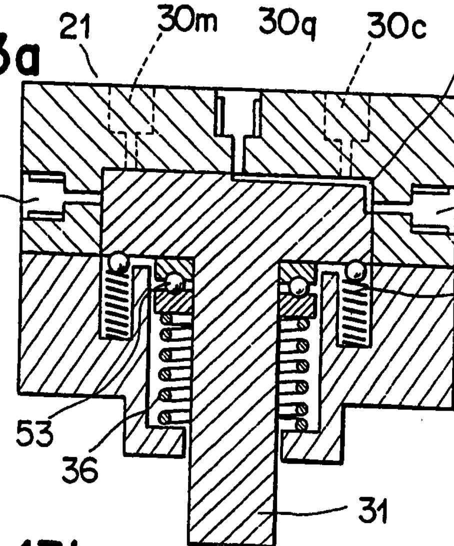


FIG. 13a

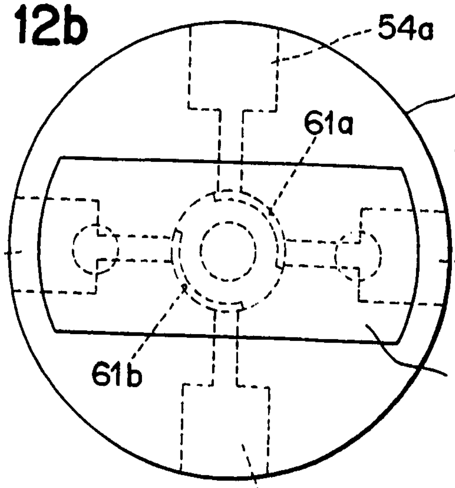


FIG. 12b

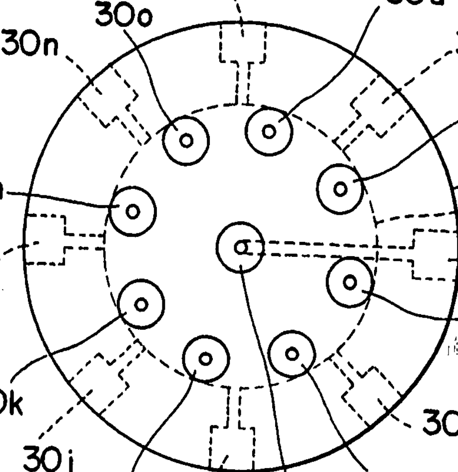


FIG. 13b

FIG. 14

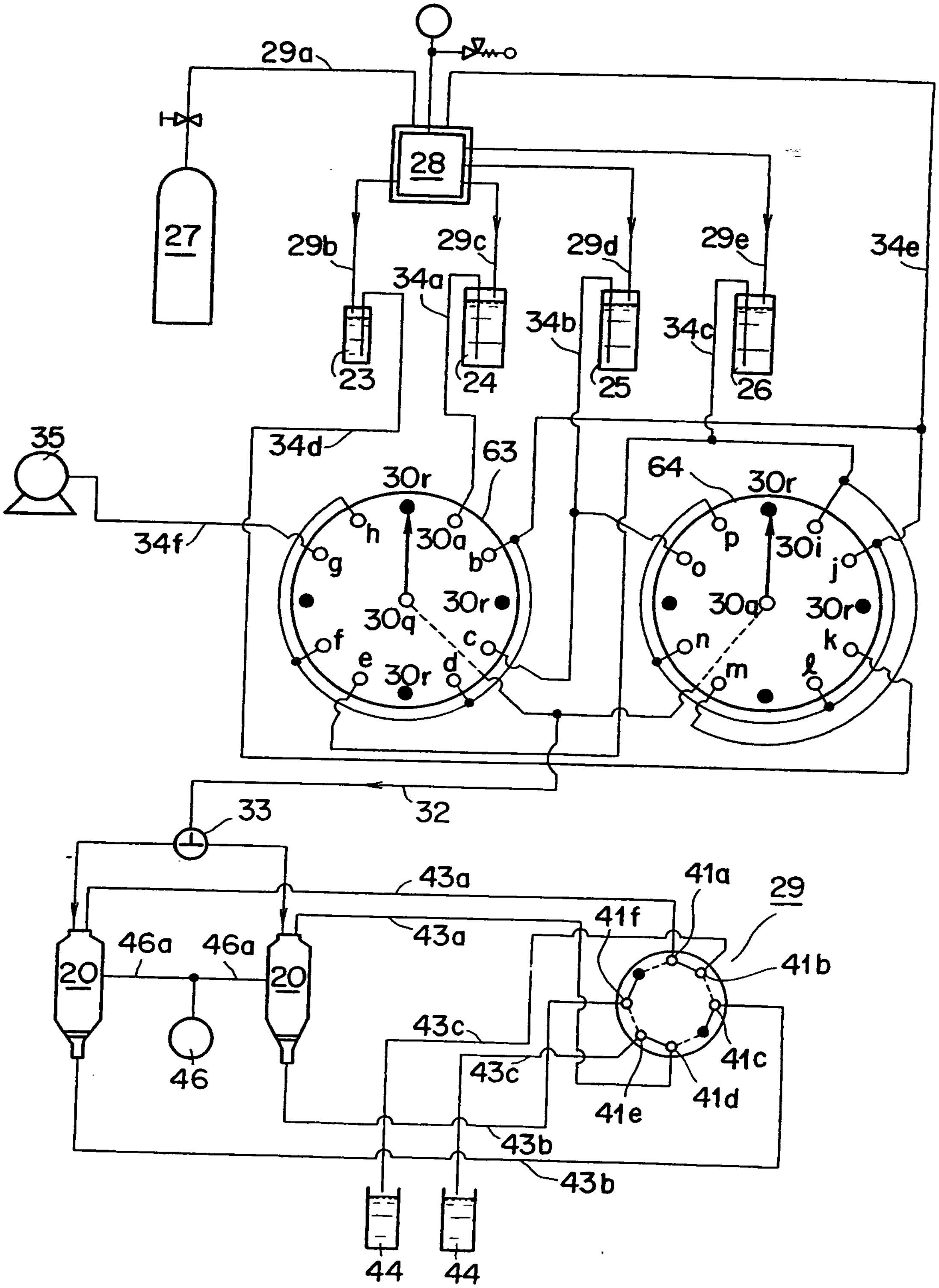

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FIG. 15a

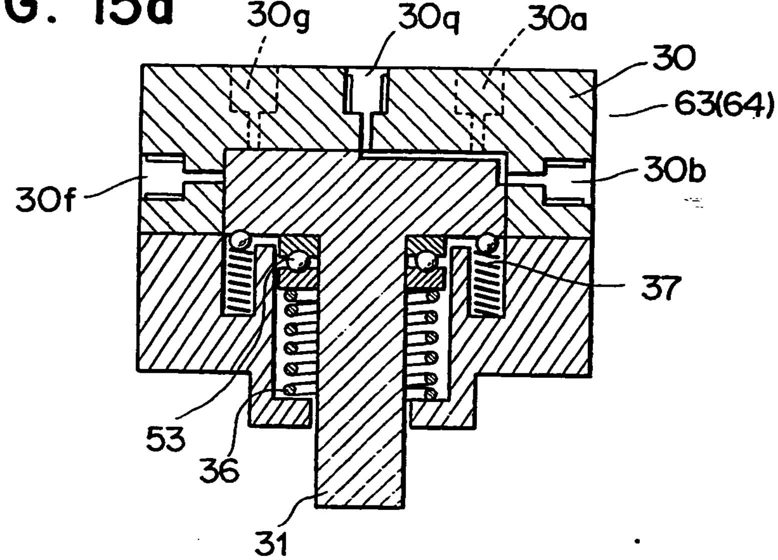


FIG. 15b

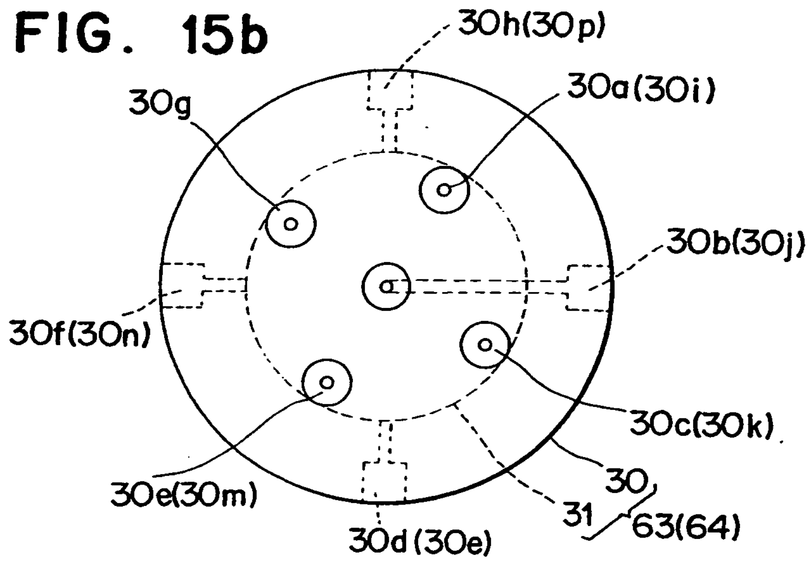


FIG. 17

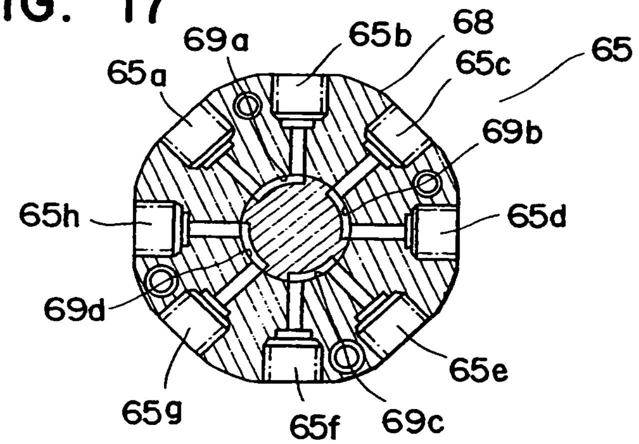
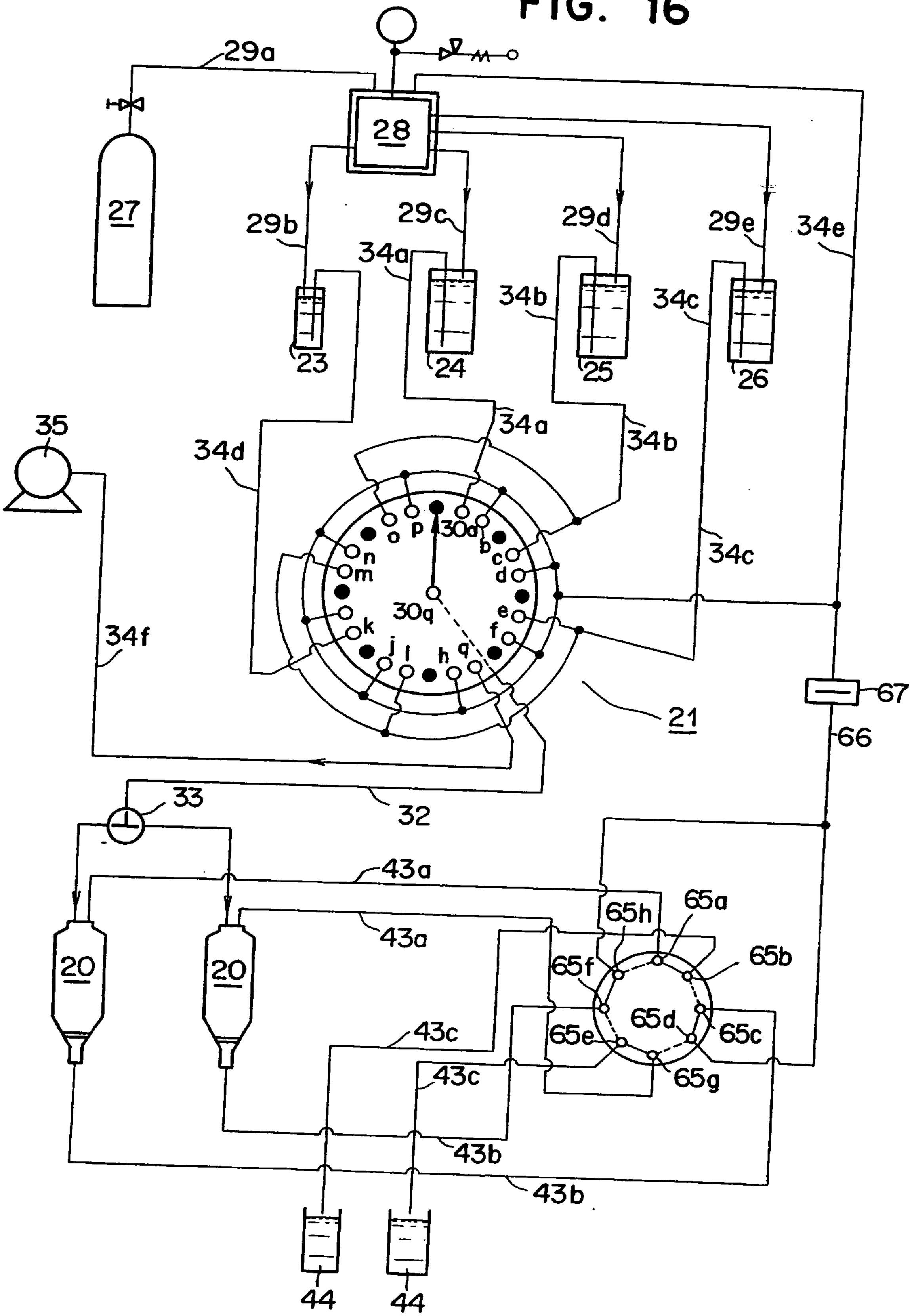


FIG. 16



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34c
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a b c d e f h q l j k m n o p
34f
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FIG. 19a

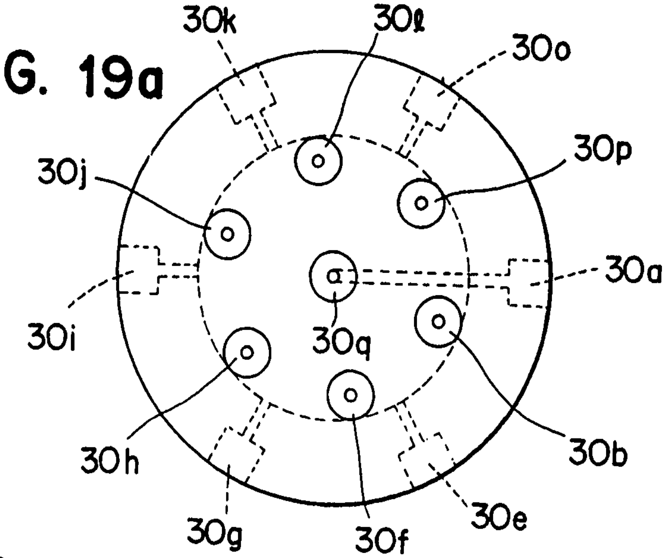


FIG. 19b

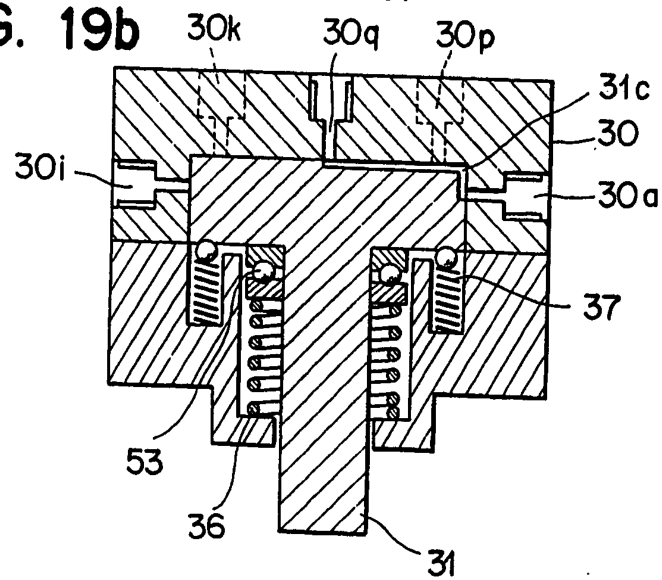
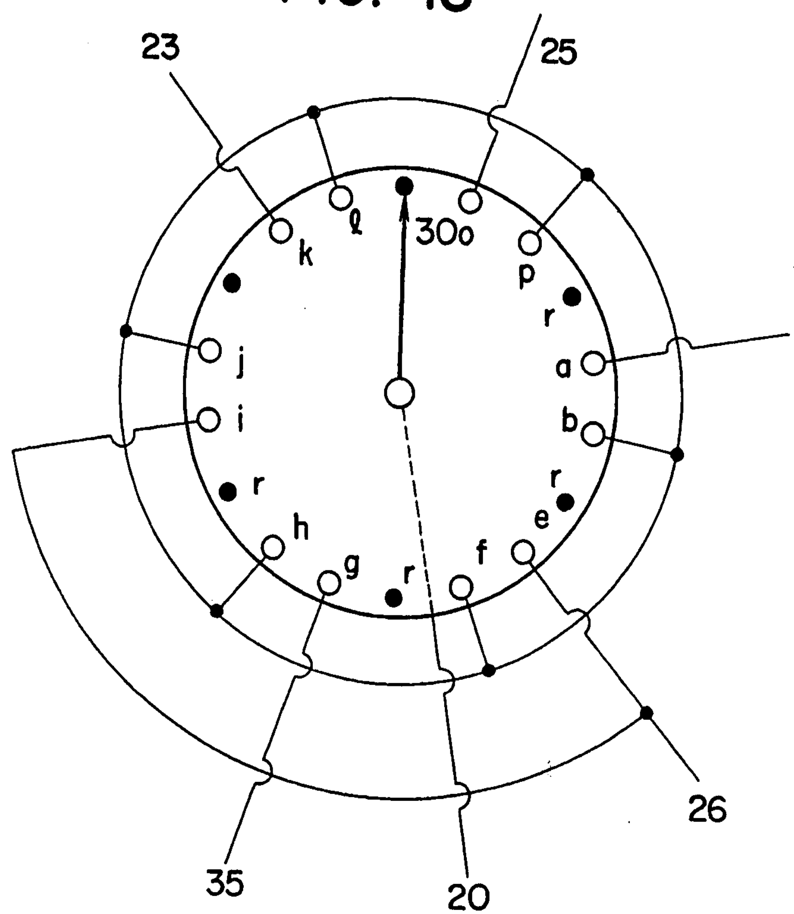


FIG. 18



SPECIFICATION

Polynucleotide synthesizing apparatus

Background of the invention

This invention relates to a polynucleotide synthesizing apparatus.

A well-known method of synthesizing polynucleotide, for example DNA (deoxyribonucleic acid) is such that a support chemically combined with nucleoside is used, and nucleotide is sequentially condensed by triphosphate method, diphosphate method, phosphite method and the like. In this synthesis method, processes such as washing → deprotection → washing → condensation reaction → washing, etc. are repeated with less variety of process. However, many tediously repeated operations are required.

Recently, various devices for synthesizing DNA have been proposed in order to eliminate tediousness of synthesis operations.

For example, a polynucleotide synthesizing apparatus is known which comprises a reactor, reagent bottles which are charged with reagents, solvents and the like required for polynucleotide synthesizing reaction, tand cocks which are provided at the passages which communicate the reagent bottles and solvent bottles with the reactor whereby the reagents and solvents and the like are successively fed to the reactor from the reagent and solvent bottles by opening and closing each cock for repeating the sequence washing → deprotection → washing etc.

However the afore-mentioned apparatus has a number of cocks so that there is a fear of misoperation. If such a misoperation takes place, all the operation should be done from the beginning, resulting in waste of all the previous operations. Although provision of solenoid valves in lieu of cocks can be carried out for automation of the apparatus, use of a number of solenoid valves involves difficulties such as high cost and complicated control.

The present applicant proposed an apparatus shown in Figure 1 in order to overcome the difficulties which is disclosed in Japanese Patent Application 58-126249. Reference numeral 1 a nitrogen cylinder; 2 a distributor; 3a to 31 passages for nitrogen gas; 4, 5, 6, 8 reagent bottles; 7 and 8 solvent bottles; 10 an eight-way change valve; 11b to 11g passages for reagents and solvents; 12 a two-way cock; 13a three-way cock; 14a to 14h passages for reagents, solvents and nitrogen gas; 15 an eight-way cock; 16 reactors; and 17 waste liquid reservoirs.

When a common port 10h is brought into alignment with the port 10b an operation knob (not shown) on the eight-way change valve 10, the reagent bottle 4 is brought into communication with the reactors 16 and 16. Under pressure of the N_2 gas which has been fed to the reagent bottle 4 from the cylinder via the distributor 2 and passage 3b, an reagent (deactivation agent) is fed to the reactors 16, 16 from the reagent bottle 4 via a passage 11b, port 10b, common port 10h, the two-way cock 12, the three-way cock 13, passages 14a and 14b. In this time the eight-way cock 15 is set at a solid-line position, that is, FEED.

When the common port 10h is brought into alignment with the port 10c by the operation knob, the reagent bottle 5 is connected with reactors 16, 16. Similarily under pressure of N_2 gas a reagent (condensation agent/solvent II solution) is fed to the reactors 16, 16 from the reagent bottle 5. Furthermore, when the common port 10h is brought into communication with the port 10d, a reagent (deactivation auxiliary agent/solvent II solution) is fed to the reactors from the reagent bottle 6. When the common port 10 is brought into communication with the port 10f, a reagent (deprotection agent/solvent I) is fed to the reactors 16, 16 from the reagent bottle 8. When the port 10h is brought into communication with port 10g the solvent II is fed to the reactors 16 and 16 from the solvent bottle 9.

When liquid is to be discharged from the reactors 16 and 16, the eight-way cock 15 is set at a dotted line position, that is, BLOW and the cocks 16a, 16a are opened.

As described above, operation of one knob makes it possible to switch the passages 11b to 11g. Misoperation may be reduced in comparison with conventional apparatus in which a number of cocks in each passage are operated to open and close and automation of the apparatus is easy.

Reviewing the operation of the eight-way change valve 10 at one condensation step, the common port 10h is sequentially switched to in the order of from the ports 10g, 10d, 10b, 10g, 10e, 10f, 10e, 10g and to 10c. Switching to the ports 10b to 10g is carried out after the common port 10d is once switched to the port 10a. That is, it is necessary to repeat the normal and reverse rotation of the eight-way change valve 10 (knob). Furthermore when the common port 10h is switched to in the order of from the ports 10b to 10g, it passes two port 10c and 10d. When the common port 10h is switched to in the order of from the port 10g to 10e, it passes one port 10f.

Therefore there is difficulty that control of a motor for driving the eight-way change valve 10 becomes complicated.

Summary of the invention

It is therefore an object of the present invention to provide a novel polynucleotide synthesizing apparatus.

It is another object of the present invention to provide a polynucleotide synthesizing apparatus in which misoperation may be reduced.

It is a further object of the present invention to provide a polynucleotide synthesizing apparatus which is simply controlled if it is automated.

In accordance with the present invention there is provided a polynucleotide synthesizing apparatus comprising reactor means; reagent and solvent bottle means which is charged with reagents and solvents required for the polynucleotide synthesis reaction; change valve means having at the inlet side thereof a plurality of reagent and solvent ports which are communicated with said reagent and solvent bottles and a common port communicated with said reactor means at the outlet side thereof, said change valve means being adapted to change the passages for communicating each of reagent and solvent bottles means with said reactor means by the rotary operation of a knob; and liquid supply means for supplying reagent and solvents from each of reagent and solvent bottles to said reactor means under pressure of inert gas, said reagents and solvent ports being disposed in order of liquid supply sequence whereby one rotation of said operation knob causes the liquid supply operation in a condensation step.

Brief description of the drawings

Figure 1 is a flow chart for showing conventional apparatus;
Figure 2 is a perspective view showing an embodiment of the present invention;
Figure 3 is a flow sheet for the Figure 2;
Figure 4 is an enlarged flow sheet showing the main part of the flow sheet of Figure 3;
Figures 5a and 5b are sectional views showing a sixteen-way change valve;
Figures 6a and *6b* are sectional views showing a six-way cock;
Figure 7 is a sectional view showing a reactor;
Figure 8 is a sectional view showing an upper plug;
Figure 9 is a partially cut away sectional view showing another embodiment of the reactor;
Figure 10 is a sectional view showing another embodiment of a sixteen-way change valve;
Figure 11 is a flow sheet showing a further embodiment in which a dual two-way cock is used in lieu of the six-way cock;
Figure 12a is a sectional view of the dual two-way cock;
Figure 12b is a plain view of the dual two-way cock;
Figure 13a is a sectional view showing another embodiment of the sixteen-way change valve;
Figure 13b is a plain view of the sixteen-way change valve;
Figure 14 is a flow sheet showing another embodiment in which two light-way change valves are used in lieu of the sixteen-way change valve;
Figure 15a is a sectional view showing a eight-way change valve used for the embodiment shown in Figure 14;
Figure 15b is a plain view showing the eight-way change valve of Figure 15a;
Figure 16 is a flow sheet showing another embodiment in which bubbling with N_2 gas can be carried out;
Figure 17 is a sectional view of six-way cock used in the embodiment of Figure 16;
Figure 18 is a schematic view showing a twelve-way change valve used in the another embodiment in which two liquid supply operations are eliminated;
Figure 19a is a plain view showing the twelve-way change valve of Figure 18; and
Figure 19b is a sectional view of the twelve-way change valve of Figure 19a.

Detailed description of the invention

Figure 2 is a perspective view showing an embodiment of the synthesizing apparatus of the present invention. Figure 3 is a flow sheet of the synthesizing apparatus.

A recess P_1 is formed at the upper portion of one end side (as viewed at the left side in Figure 2) of a front panel P of the apparatus body A. Reactors 20, 20 which are provided in the recess P_1 are supported by arms 46a of shakers 46 as shown in Figure 3.

An operation knob 22 for sixteen-way change valve 21 (refer to Figures 3, 4 and 5a and b) corresponding to eight-way change valve 10 shown in Figure 1 is provided on the front panel P.

Recesses A_1 and A_2 are formed at one side of the apparatus body. A reagent bottle 23 which is charged with a deactivation agent and deactivation auxiliary agent is disposed in the lower recess A_1. A regent bottle 24 charged with a deprotection agent (detryt yl agent)/ solvent I solution, a solvent agent bottle 25 charged with solvent I and a solvent bottle 26 charged with solvent II are disposed in the upper recess A_2.

In this embodiment, a reagent bottle 5 (condensation agent/solvent II solution) is eliminated so that deactivation agent and deactivation auxiliary agent are charged in the same reagent bottle 23 as described above.

A small nitrogen cylinder 27, a distributor 28, a six-way cock 29 and the sixteen-way change valve 21 etc. are disposed inside of the apparatus body A.

The N_2 gas in the nitrogen cylinder 27 is fed via a passage 29a to the distributor 28 where it is shunted. The gas is then fed to the reagent bottles 23 and 24 and the solvent bottles 25 and 26 via passages 29b, 29c, 29d and 29e. Reagent and then solvent are fed to the reactors 20, 20 from bottles 23 to 26 under the pressure of the N_2 gas.

The sixteen-way change valve 21 is disposed between the reagent bottles 23, 24, solvent bottles 25, 26 and the reactors 20, 20.

The sixteen-way change valve 21 which is a feature of the present invention will be described with reference to Figures 4 and 5a and 5b.

The sixteen-way change valve 21 comprises a valve body 30 and a rotor 31 which is rotatably mounted on the valve body 30.

The valve body 30 is formed with inlet ports 30a to 30p and an outlet common port 30q. A peripheral recess 31a and a longitudinal recess 31b are formed on the outer periphery of the rotor 31. Rotation of the rotor 31 causes the common port 30q to successively communicate with ports 30a to 30p via the peripheral recess 31a and the longitudinal recess 31b.

The common port 30q is communicated with the upper portion of the reactors 20, 20 via a passage 32 and a three-way cock 33 at the length thereof.

The reagent and solvent ports 30a, 30c, 30e, 30i, 30k, 30m, 30o of the ports 30a to 30p are communicated with the reagent bottles 23, 24 and the solvent bottles 25 and 26. The gas ports 20b, 30d, 30f, 30h, 30j, 30l, 30n, 30p are communicated with the distributor 28 via a passage 34e.

The reagent and solvent ports 30a, 30c, 30e, ... are disposed in an liquid supplying operation order. The gas ports 30b, 30d and 30f ... and a liquid supplying stop portion 30r are disposed therebetween.

Accordingly, one rotation of the rotor 31 causes liquid supply operation in the order of from such as supply of deprotection agent/solvent I from the reagent bottle 24, N_2 gas, stop of liquid supply, supply of solvent I from the solvent bottle 25, N_2 gas and to stop of liquid supply. In other words, one rotation of the rotor 31 completes the liquid supply and discharge operation in one condensation step.

The position for stopping the liquid supply has a same function as two-way cock 12 shown in Figure 1. The two-way cock 12 is eliminated in the present invention.

Port 30g is communicated with a vacuum pump 35 via a passage 34f. When the port 30q is switched to the port 30g, the reactors 20, 20 are communicated with the vacuum pump 35 for simultaneously effecting boiling and dehydration.

When automization of the apparatus is desired, the rotor 31 may be linked with a stepping motor or servo-motor and the motor is sequence-controlled in accordance with a program stored in a control device. In this case passing over one or two ports as is done by the apparatus of Figure 1 is not effected since the reagent and solvent ports 30a, 30c, 30e ... are disposed in an order of liquid supply operation. This simplifies the motor control. A drum type sequencer may be used for driving the motor.

The rotor 31 is tapered so that it has a reduced diameter at one end (as viewed at left side in Figure 5b). A spring 36 is disposed between the valve body 30 and the rotor 31 for biasing the rotor in a direction to one end. Therefore there is no possibility to form a gap between the valve body 30 and the rotor 31. Ports 30a to 30p are not communicated with the common port 30q except for the peripheral recess 31a and longitudinal recess 31b.

A click mechanism 37 is provided at one end of the valve body 30. The click mechanism 37 comprises a disc plate 38 which is secured to the one end of the rotor 31 and slidably connected with the end face of the valve body 30 and a ball 40 which is disposed in a hole 30′ formed on the one end face of the valve body 30 and is biased upon the slide face of the disc plate 38 by a biasing force of a spring 39. The slide face of the disc plate 38 is formed with a recess 38a corresponding to ports 30a to 30p and the liquid supply stopping position 30a. Positioning of the rotor 31 is accomplished by the engagement of a ball 40 with the recess 38a.

The afore-mentioned six-way cock 29 comprises a cock body 41 and a rotor 42 which is rotatably mounted on the cock body 41.

The cock body 41 is provided with ports 41a to 41f. The rotor 42 is provided with a peripheral recesses 42a to 42d for communicating the ports 41a to 41f with each other.

The connection relationship between ports 41a to 41f and reactors 20, 20 is shown in Figure 3. That is, ports 41a and 41d are communicated with the upper portion of the reactors 20, 20 via passages 43b and 43b. The ports 41c and 41f are communicated with the bottom portion of the reactors, 20, 20 via passages 43b, 43b. The ports 41b and 41e are communicated with waste liquid reservoirs 44, 44 via passages 43c and 43c.

Ports 41a and 41d are communicated with ports 41b and 41e via the peripheral recesses 42a and 42c and ports 41c and 41f are closed in a position represented by a solid line shown in Figure 3, that is, FEED condition. When the rotor 42 is rotated to move the peripheral recesses 42a to 42f to a position represented by a dotted line of Figure 3, the ports 42b and 41e are brought into communication with the ports 41c and 41f and the ports 41a and 41d are rendered closed to establish a BLOW condition.

The six-way cock 29 is also provided with a click mechanism 45. The click mechanism 45 serves to position the peripheral recesses 42a to 42d at the positions represented by solid and dotted lines in Figure 3 and also serves to position the recesses at positions intermediate between positions represented by solid and dotted lines. When the recesses are positioned at the intermediate position, the ports 41a to 41f are fully closed.

Six-way cock 29 functions as a combination of a six-way cock 15 shown in Figure 1 and the cocks 16a and 16a of the reactors 16, 16. Therefore the reactors 20, 20 are not provided with any cock. The rotor 42 of the six-way cock 29 may be linked with a stepping motor as is done in case of a sixteen-way change valve. Automization may be accomplished by driving the motor in accordance with a program stored in a control system.

On the front panel P, there are provided an operation knob 41 for the six-way cock 29, an operation knob 48 for the three-way knob 33, an operation knob 49 for a timer (not shown) of the shaker 46, an N_2 gas pressure meter 50 connected with the distributor 28, and a power switch 51 etc.

Attachments 23a to 26a for the necks of the bottles 23 to 26 which are not shown in detail are provided at the ceiling of the recesses A_1 and A_2.

Figure 7 is an enlarged sectional view showing the reactor 20. The reactor 20 comprises a reactor body 55 and an upper and lower plugs 56 and 57.

The upper and lower plugs 56 and 57 are attached to the reactor body 55 by mounting the flanges 56a and 57a upon the flanges 55a and 55b formed at the upper and lower ends of the reactor body 55 by means of a fastening means such as clip respectively.

The reactor body 55 is made of glass and is formed with the stepped portions 55c and 55d in the upper and lower openings respectively. The stems 56b and 57b of the upper and lower plugs are inserted into the body until they are brought into contact with the stepped portions 55c and 55d respectively.

The upper and lower plugs 56 and 57 are made of a material which will not react with the afore-mentioned reagent and solvent, etc. and is elastically deformable, such as fluorosilicon rubber fluorine rubber, copolymer of propylene hexafluoride and ethylene tetrafluoride and the like.

The plugs 56b and 57b has an outer diameter slightly larger than an inner diameter of the upper and lower openings so that the outer surface of the plugs 56a and 57a are in close contact with the inner surface of the upper and lower openings to keep fluid-tight relationship.

The upper plug 56 is provided with a cock 58 through which a stock material is charged. The plug are provided with passages 56c and 56d which are communicated with passages 32 and 43a respectively.

The lower plug 57 is formed with a passage 57c which is communicated with a passage 43b.

O-rings may be disposed in peripheral recesses 56b′ and 57b′ on the stems 56b and 57b respectively.

In the present invention, a filter may be provided at the side of a liquid supply portion of the reactor into which reagent and solvent and the like are introduced. The liquid supply portion may be provided with an admission passage for introducing reagent and solvent and the like to the liquid supply portion via the filter and a purge passage for purging the gas from the reactor via the filter (pressure purge passage).

By forming in this manner, reagent and the like may be prevented from being purged from the introduction passage directly via the purge passage and the valves along the passages in the apparatus body may be protected.

The present embodiment will be described with reference to Figure 9.

Filters 124 are sandwiched between the stepped portions 121a, 121a of the body 55 and the upper and lower plugs 56 and 57. The filters 124 are made of a material such as tetrafluoroethylene, polypropylene, polyethylene and the like which a can trap the support charged into the body 55 and is insoluble in the afore-mentioned solvent and has a permeability to the solvent. One end portion of the upper plug 56 is formed slightly larger than inner diameter of the body 55. A peripheral recess 122a is formed on the outer periphery of the end portion. The end face is formed with a semispherical recess 122b so that the end portion is elastically deformed in an inner diameter direction of the body 55 to bring into close contact with the inner periphery of the body to keep an air-tight relationship with a body 55.

An admission and purge passages 122c and 122d extending in an axial direction are formed in the upper plug 56 in a close relationship with each other. The admission and purge passages 122c and 122d are connected with the passages 120a and 120b respectively.

A tube 122e extending through the filter 124 is connected with one end of the admission passage 122c so that an admission inlet is positioned inside of the filter 124. On the contrary to this, the inlet of the purge passage 122d is positioned in the recess 122b, that is, outside of the filter 124. Accordingly, although the admission and purge passages 122c and 122d are formed in a closed relationship, the solvent and the like which has been introduced from the admission passage 122c will enter the body 55 without directly entering the purge passage 122d since the inlets of admission and purge passages are apart from each other.

The N_2 gas and the solvent and the like which is over-flown from the body 55 when pressure is purged is discharged from the purge passage 122d to the passage 120b via the filter 124. Accordingly there is no possibility that the cock along the passage 120b will be broken by foreign material.

An end portion of the lower plug 57 which is inserted into the body 55 is formed with a peripheral recess 123a and a recess 23b. The end portion is brought into close contact with the inner periphery of the body 55 due to elastic deformation to keep air tight relationship. A purge passage 123c axially extending is formed in the lower plug 57 and is communicated with the passage 120c.

Flange portions 121b and 121b are provided at the opposite ends of the body. Flange portions 122f and 123d are provided at the mid point between the upper and lower plugs 56 and 57. The upper and lower plugs 56 and 57 may be secured to the body 55 by linking the flange portions 121b and 121b with the flange portions 122f and 123d respectively. Provision of taper at the inner periphery at the ends of the body 55 makes it easier to insert the upper and lower plugs 56 and 57.

The reactors 20 may be disposed in a recess P_1 formed on the apparatus casing so that they can be directly viewed from outside. The reactors 20 are provided with heaters (not shown). The heater is in the form of C in section and comprises a heating plate and a thermal sensor embedded in a cylindrical body.

The cylindrical body is made of silicon rubber and has an axially extending slit. The reactors can be viewed through the slit so that the liquid supply is observed. Colouring the cylindrical body and provision of aluminium foil on the inner surface made it easier to observe the liquid supply to the reactor 20 (made of glass so that the inside thereof can be viewed).

The heating plate is made of a resistor coated with silicone rubber. The plate is embedded in the vicinity and along the inner peripheral surface of the cylindrical body for surrounding the reactor 20. The heating plate has such a size that it can effectively heat the reaction zone of the reactor 20.

The thermal sensor is made of platinum resistor and is positioned in the vicinity of the heating plate for detecting the temperature of the heating plate. Chromel-alumel thermocouple and thermister may be used in lieu of the platinum resistor.

The inner diameter of the cylcindrical body is made slightly larger than the outer diameter of the reactor 20. The cylindrical body is attached to the reactor 20 under the condition that it is flexed, that is, an elastic recovery force acts. Therefore the sylindrical body is brought into close contact with the reactor 10 to enhance the thermal conductivity.

Operation for synthesizing DNA by using the aforementioned apparatus will be described.

A support which is combined with nucleoside is charged into the reactors 20 and 20. The reagent bottles 23 and 24 and solvent bottles 25 and 26 are set at the attachments 23a to 26a. The support is then swelled by solvent I.

The support may be organic material, such as polystylene, polyester, polyamide polyurethane, polypeptide and inorganic material such as silica, alminosilicate, borosilicate. Polystylene and silica are typically used as the support.

After completion of the preparation in such a manner, detritylation is carried out. At this detritylation step, the common port 30q of the sixteen-way change valve 21 is switched to the port 30a for feeding deprotection agent/solvent I solution from the reagent bottle 24 to the reactors 20, 20. When the supplied solution reaches at a given amount, the port 30q is switched to the port 30b. The N_2 gas expels the residual deprotection agent/solvent I solution remaining in the passage 32 to the reactors 20, 20. The valve 21 is switched to the position 30a for stopping liquid supply. The shaker 46 is then activated if necessary. After a passage of a predetermined period of time the six-way cock 29 is switched to BLOW (at position represented by a dotted line in Figure 3). The valve 21 is switched to the port 30b so that the deprotection agent/solvent I solution is discharged from the reactors 20, 20 to the waste liquid reservoirs 44, 44 via the passage 43b, ports 41c, 41b, 41e, 41f and the passage 43c under the pressure of N_2 gas. After the discharge of waste liquid has been completed, the port 30q is brought into communication with the port 30c and the six-way cock 29 is set to FEED (at position of dotted line of Figure 3).

Deprotection agent may be proton acid such as benzen sulfonic acid, dichloroacetic acid, trichloroacetic acid, trifluoroacetic acid and Lowis acid such as zinc bromide, aluminium chloride, titanium tetrachloride.

Solvent I may include dichloroethane, chloroform, acetonitrile, nitromethane, dioxane, tetrahydrofuran. If necessary, said solvent I may be used together with alcohol such as methanol, ethanol, isopropanol, n-buthanol. Then washing with solvent I is carried out. At this washing step the sixteen-way change valve 21 is switched in the order of from ports 30c, 30d and to 30r. Solvent I is fed from the solvent bottle 25 to the reactors 20, 20 without leaving it in the passage 32. After the shaker 46 is activated for a given period of time, if necessary, the six-way cock 29 is set at BLOW. The sixteen-way change valve 21 is switched to in the order of from port 30d to 30r to discharge waste solvent I. Thereafter the six-way cock 29 is set at FEED.

Detritylation step (I) and washing step (II) may be repeated plural times if necessary.

Thereafter washing with solvent II is carried out. At the washing step (III), the sixteen-way change valve 21 is switched to ports in the order of from 30e, 30f and to 30r to feed the solvent II from the solvent bottle 26 to the reactors 20, 20 without leaving the solvent II in the pqassage 32. After the shaker 46 is activated for a predetermined period of time the six-way cock 29 is set at BLOW. The sixteen-way change valve 21 is switched to the port 30f to 30r. The six-way cock 29 is set at FEED after completing the discharge of the solvent II. Solvent II may include pyridines such as pyridine, picoline, lutidine, and quinoline, isoquinoline, oxazole and may be conveniently used together with ethers such as tetrahydrofuran and dioxane.

The washing step (III) may be repeated plural times if necessary.

After this, stock is injected. At this stock injection step (IV) the cock 58 (refer to Figure 70 is opened. Stock solution in which a monomer salt having a following structure is dissolved with solvent II is injected from the upper portion of the reactors 20, 20.

$$\mathrm{DMTr.\,(R1)dB-O-\underset{\displaystyle |\atop \displaystyle O-\phi Cl}{\overset{\displaystyle O\atop \displaystyle \|}{P}}-O^{\ominus}N^{\oplus}H(R2)_3}$$

wherein

R1 represents a protection group (benzoyl group and the like);

R2 represents an alkyl group; and

B represents a nucleic acid base such as adenine (A), quanine (G), cytosine (C), thiamine (T).

Dimer salt may be used in lieu of monomer salt.

The water content in the stock is then simultaneously boiled and dehydrated under a reduced pressure. At this dehydration step (V) the six-way cock 29 is fully opened. The sixteen-way change valve 21 is switched to the port 30g and the vacuum pump 35 is operated. This evacuates the reactors 20, 20 to simultaneously boil and dehydrate the water content which has been dissolved into the solvent II from the stock. After dehydration is completed, the valve 21 is switched to the port 30h for charging N_2 gas. Thereafter the valve

21 is switched to the position for stopping liquid supply 30r and the six-way cock 29 is set at FEED. Thereafter condensation is carried out. At this condensation step (VI) condensation agent/solvent II solution is injected from the upper portion of the reactors 20, 20. If necessary, the shaker 46 is activated and the reactors 20, 20 is heated by a heater (not shown). After the completion of condensation reaction the shaker 46 and heater are deenergized. The six-way cock 29 is set at BLOW and the sixteen-way change valve 21 is switched to port 30h and 30r successively. After the completion of liquid discharge the six-way cock 29 is set to FEED.

Condensation agent may be for example, 2.4.6-triisopropylebenzensulfonyltetrazoride, P-nitrobenzensulfonyltriazoride, benzensulfonyltriazoride, 2.4.6-trimethylebenzensulfonyltriazoride, 2.4.6-trimethylbenzensulfonylnitrotriazoride, 2.4.6-trimethylebenzensulfonyltetrazoride, 2.4.6-triisopropylebenzensulfonylchloride, 2.4.6-trimethylebenzensulfonylchloride.

Then washing with solvent II is accomplished. At the washing step (VII) the sixteen-way change valve 21 is switched to in the order of from ports 30i, 30j and from 30r successively. The operation is similar to the washing step (III).

The washing step may be repeated plural times if necessary.

Capping is then carried out. At this capping step, the sixteen-way change valve 21 is switched to in the order of from ports 30k, 30l and to 30r successively so that deactivation agent/deactivation auxiliary agent/solvent II solution is fed to the reactors 20, 20 via the passage 32 from the reagent bottle 23. After the shaker 46 in activated for a given period time, the six-way cock 29 is set and the sixteen-way change valve 21 is switched to in the order of from ports 30l and to 30r successively for discharging waste liquid. After the completion of the discharge of waste liquid the six-way cock 29 is set to FEED.

Deactivation agent may include acetic anhydride, phenyl isocyanate, diethylemonotriazorophosphite and if necessary base such as N,N-dimethyleaminopyridine, N,N-diethyleaminopyridine, N,N-dimethyleaminopicoline may be used as a reaction promotor.

Washing with solvent I is then carried out. At this washing step (IX), the sixteen-way change valve 21 is switched to in the order of from the ports 30m, 30n and to 30r successively. The operation is similar to that of step (III).

Washing with solvent (I) is then carried out. At this washing step (X) the sixteen-way change valve 21 is switched to in the order of from the ports 30o, 30p and to 30r successively. The operation is similar to that of step (III).

Washing steps (IX) and (X) may be repeated plural times if necessary.

The afore-mentioned steps (I), (II), (III), (IV), (VII). (IX) and (X) is repeated plural times if necessary. In this time, only the six-way cock 29 may be operated. For example, if the step (II) is repeated, the six-way cock 29 is switched in the order of from FEED, BLOW, FEED and to BLOW successively while the valve 21 is set at the port 30c. This enables reduction in the number of operations.

Repetition of steps (I) to (X) causes nucleotide chains to successively condensate.

The number of valve operations may be reduced since the sixteen-way change valve 21 and the six-way cock 29 in the present embodiment also serve as the two-way cock 12, cocks 16a, 16a of the reactors 16 and 16 shown in Figure 1.

For example, in order to carry out washing with solvent II in the apparatus shown in Figure 1, (1) the eight-way change valve 10 is set to the port g, (2) the two-way cock 12 is closed, opened and closed, (3) the valve is set to the port (10a), (4) the two-way cock 12 is closed, opened and closed, (5) the eight-way cock 15 is set at in the order of from FEED to BLOW, (6) the cock 16a is closed, opened and closed, (7) the eight-way cock 15 is set at BLOW to FEED.

On the other hand, in the present embodiment (1) the sixteen-way change valve 21 is switched to in the order of from the ports 30e, 30f and to 30r, (2) the six-way cock 29 is set at in the order of from FEED to BLOW, (3) the valve is switched to in the order of from the ports 30f to 30r, (4) the six-way cock 29 is set at in the order of from BLOW to FEED.

This means that the number of the valve operations may be reduced by three times in the present embodiment.

Figure 10 shows another embodiment of the sixteen-way change valve 21. In the present embodiment, the ports 30a to 30p is brought into communication with the common port 30q only when the longitudinal recess 31b is aligned with the ports 30a to 30p by pressing the rotor 31 in a direction of an arrow B in Figure 10 against the biasing force of the spring 52. That is, reagent, solvent, N_2 gas may be supplied to the reactors 20, 20 while the rotor 31 is pressed.

Figure 11 shows another embodiment in which a dual two-way cock 54 is used in lieu of the six-way cock 29. In accordance with the dual two-way cock, the ports 54a and 54b are connected with the bottom of the reactors 20, 20 via the passages 43b, 43b respectively and the ports 54c,54d are connected with the waste liquid tanks 44, 44 via the passages 43c and 43c. Solid line represents FEED position and dotted line represents BLOW position.

In the present embodiment, the number of valve operations may be furthermore reduced than the former embodiment. For example, when washing with solvent II is carried out. (1) The sixteen-way change valve 21 is switched to in the order of from the ports 30e, 30f and 30r, (2) the dual two-way cock 54 is set at in the order of from FEED, BLOW and to FEED. Therefore the number of valve operations may be reduced by 2 than that of the former embodiment and may be reduced by 5 than that of the apparatus shown in Figure 1. Accordingly the synthesizing operation time can be shortened. Since the inner pressure in the reactors 20, 20

becomes 1 kg/cm^2 which is substantially equal to the N_2 gas for feeding the liquid when a half of the reactors 20, 20 is filled with liquid in the present embodiment, liquid supply is automatically stopped by the inner pressure without switching to the liquid supply stopping position 30r so that the number of valve operations may be furthermore reduced.

The afore-mentioned dual two-way cock 54 comprises a body 60, a rotor 61 and a click mechanism 62 as shown in Figures 12a and 12b. The body 60 is provided with ports 54a to 54d. Recesses 61a and 61b which communicate the ports 54a to 54d with each other are formed on the periphery of the rotor 61.

The sixteen-way change valve 21 may be formed as shown in Figures 13a and 13b. In this case, a common port 30q is formed at the center of the end face of the valve body 30. Reagent and solvent ports 30a, 30c ... are formed so that they surrounds the ports. Gas ports 30b, 30d — are radially formed on the periphery of the valve body 30. The rotor 31 is formed with a common recess 31c for communicating the common port 30q with the ports 30a to 30p so that the recess extends from the end face to the periphery of the rotor 31.

Figure 14 shows a case in which two eight-way change valves 63, 64 are used in lieu of the sixteen-way change valve. One eight-way change valve 63 is formed with the first half ports 30a to 30h of the afore-mentioned ports 30a to 30p in order of liquid supply and the other eight-way change valve 64 is formed with the second half ports 30i to 30p in order of liquid supply. Each of eight-way change valves 63, 64 is formed with a common port 30q.

In this embodiment, after the steps (I) to (VI) are accomplished by changing one eight-way change valve 63, the steps (VII) to (X) are accomplished by switching the other eight-way change valve 64.

Spacings among the ports 30a through 30p may be made larger by assigning the ports to two eight-way changed valves 63 and 64 so that sealing properties are readily assured and the reliability of the valve may be enhanced.

Figures 15a and 15b show the eight-way change valves 63, 64. The basic structure is identical with the sixtee-way change valve 21 while the spacings among the ports 30a to 30p are larger.

Figure 16 shows a case in which a eight-way cock 65 is used in lieu of the six-way cock 29 so that bubbling with N_2 gas may be carried out. As shown in Figure 16, the ports 65g and 65h are connected with a branch passage 66 which branches from the passage 34e. The branch passage 66 is provided with an orifice 67 for passing a necessary amount of N_2 gas. A capillary or needle valve may be used in lieu of the orifice. The ports 65a to 65f are connected with the reactors 20, 20 and the waste liquid reservoirs 44 and 44 as is similar to the six-way cock 29.

In the present embodiment, a small amount of N_2 gas which has passed the orifice 67 is blown from the bottom of the reactors 20, 20 to effect bubbling when the eight-way cock 65 is set at FEED. Accordingly the shaker 46 my be eliminated resulting in a light weight and compact apparatus and reduction in manufacturing cost.

The branch passage 66 may be provided with a push valve for effecting bubbling while the push valve is pressed.

In the present invention, the passages for supplying reagent, solvent and the like which are connected with the top of the reactors 20, 20 as shown in Figure 16 may be connected with the bottom of the reactors and inert gas may be supplied to the bottom of the reactors via a flow rate regulator which regulate the flow rate of the gas to an value required for bubbling.

In such a manner supply of reagent into the bottom of the reactors causes agitation simultaneously with the liquid supply. Accordingly, sufficient contact of the reagent and the like with the support may be accomplished and the operation time may be shortened compared with a case in which the reagent and the like is supplied to the top of the reactors. There is no possibility that the reagent and the like be overflown from the reactors when bubbling is effected and time is not consumed.

Figure 17 shows the afore-mentioned eight-way cock 65. As shown in Figure 17 the ports 65a to 65h are disposed at the body 68 in a radial manner. The rotor 69 is rotatably provided at the center of the ports. The rotor 69 is formed with peripheral grooves 69a to 69d for communicating the ports 65a to 65h with each other.

Figure 18 shows a case in which a twelve-way change valve 70 is used in lieu of the sixteen-way change valve 21. In the present embodiment, the afore-mentioned ports 30c, 30d, 30m, 30n are eliminated because the deprotection agent used at detritylation step and deactivation agent and deactivation auxiliary agent used at capping step are dissolved to solvents I and II respectively. In the former embodiment, washing step is provided in which the reactor is washed with solvent I for removing the deprotection agent (detrityl agent) remaining in the reactors after the detritylation step (I) while the washing step (II) is followed by a washing step (III) using solvent (II) in the present embodiment. Accordingly deprotection agent may be solved and removed at washing step if the washing step (II) is eliminated.

The capping step (VII) is followed by the washing step (IX) at which the reactors are washed with solvent II for removing the deactivation auxiliary agent remaining in the reactors 20, 20. Since the washing step (IX) is followed by a washing step (X) for washing the reactors 20 with solvent I, the deactivation agent and deactivation auxiliary agent may be dissolved and removed at the step (X) even if the washing step (IX) is eliminated.

Figures 19a and 19b show the afore-mentioned twelve-way change valve 70. Basic structure thereof is identical with the sixteen-way change valve 21 shown in Figures 12a and 12b except for that the spacings among the ports 30a 30p becomes larger by a length where the ports 30c, 30d, 30m, 30n have been provided.

It is easy to assure sealing performances at ports 30a to 30p and the reliability of valve may be enhanced.

In the afore-mentioned embodiments, two reactors 28, 20 have been disclosed. The invention is not limited to this case. There may be provided one reactor or more than two reactors. The structure of the six-way cock 29 and eight-way cock 65 is different from those of the afore-mentioned embodimens when there is provided one reactor or there are provided more than two reactors.

There has been disclosed a case in which the stock is charged into the reactors by opening the cock 58. Stock solution having stock dissolved in solvent which is charged into a bottle may be fed to the reactors under the pressure of N_2 gas.

Two or three solvents are conventionally used at each washing step and the reactors are successively washed in a repeated manner. This involves sophisticated operation and an extended period of operation time. There is also a problem that the apparatus can not be made small due to increase in the number of solvents. The present invention has overcome the problem by using one solvent which is capable of dissolving impurity existing in a product produced in the previous step and which can be used at the next step. That is, sufficient effect may be obtained by washing with one solvent.

The synthesizing apparatus of the present invention can be used for the synthesis of RNA (ribonucleic acid) as well as DNA.

In accordance with the present invention, the reagent and solvent inlet ports of the change valve which change passages for communicating the reagent and solvent bottles with the reactors by rotating the operation knob are disposed in order of liquid supply operation. One rotation of the operation knob completes a liquid supply and discharge operation. Therefore the operation is very simple and the misoperation may be reduced.

Automation of the apparatus does not require a number of solenoid valves. For example only a stepping motor or servo-motor is required to automate the apparatus. Its control is simple. It is easy to convert the apparatus for automation.

CLAIMS

1. A polynucleotide synthesizing apparatus comprising reactor means;
reagent and solvent bottle means which is charged with reagents and solvents require for the polynucleotide synthesis reaction;
change valve means having at the inlet side thereof a plurality of reagent and solvent ports which are communicated with said reagent and solvent bottles and a common port communication with said reactor means at the outlet side thereof, said change valve means being adapted to change the passages for communicating each or regent and solvent bottles means with said reactor means by the rotary operation of a knob; and
liquid supply means for supplying reagents and solvents from each of reagent and solvent bottles to said reactor means under pressure of inert gas, said reagent and solvent ports being disposed in order of liquid supply sequence whereby one rotation of said operation knob causes the liquid supply operation in a condensation step.

2. The apparatus as defined in Claim 1 in which a passage for introducing said inert gas to said reactor means is provided with a branch passage having a flow rate restriction means through which inert gas glows at a flow rate required for bubbling.

3. The apparatus as defined in Claim 1 in which a liquid supply passage is connected with the bottom of said reactor means so that said inert gas is supplied from the bottom of said reactor means via said flow rate regulating means at a flow rate required for bubbling.

4. The apparatus as defined in Claim 1 in which a filter is provided at the liquid supply portion of said reactor means and an admission passage for introducing the reagents and solvents to the reactor means through said filter and a purge passage for introducing gas and the like generated in the reactor means to the outside of the reactor means via the filter are provided at the liquid supply portion.

5. The apparatus as defined in Claim 1 in which said reactor means is disposed so that it can be viewed and a heater having a heating element is embedded in a main body so that the heating element surrounds the reactor means, said main body having a slit through which said reactor means can be directly viewed and said main body being formed to grip the reactor means.

6. The apparatus as defined in Claim 1 in which said change valve means for changing the passages communicating the reagent and solvent bottles with reactor means by the rotation of the operation knob includes a plurality of valves.

7. The apparatus as defined in Claim 1 in which said reactor means includes a plurality of reactors.

8. The apparatus as defined in Claim 1 in which said reactor means includes a single reactor.

9. The apparatus as defined in Claim 1 in which one solvent is used which can dissolve impurity in the product produced in the previous step for each step and is usable in next step.

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